Complex Cadherin Expression in Renal Cell Carcinoma

Toru Shimazu, Laurence A. Giroldi, Pierre Paul Bringuier, Egbert Oosterwijk, and Jack A. Schalken

Urological Research Laboratory, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands

Abstract

E-cadherin is an intercellular adhesion protein expressed by most epithelia. Decreased expression of E-cadherin correlates with tumor aggressiveness in most carcinomas. In renal cell carcinoma (RCC), however, this correlation is not well established and the prevalence of negative tumors is higher than in other carcinomas. Our immunofluorescence study of α-catenin expression in 20 RCC cell lines revealed a typical honeycomb staining pattern in all of the lines, whereas only six expressed E-cadherin. This suggested that other cadherins are expressed in RCC lines. Indeed, immunoprecipitation with an anti-α-catenin antibody resulted in coprecipitation of proteins of M, 125,000–135,000. Using Western blot, these proteins react with a pan-cadherin antibody. To identify these cadherin related proteins, RT-PCR using degenerated primers and sequence comparisons was carried out. We then assessed the expression of the identified cadherins. N-cadherin mRNA was present in all cell lines; and cadherin 6 mRNA was seen in 16 lines. Cadherin 11 (mRNA) and E-cadherin (protein) were expressed in five and six lines, respectively. A cadherin 4 transcript was observed in only one line, whereas no P-cadherin protein could be detected. Expression of the four main cadherins was also found in normal kidney (two samples tested) and RCC specimens (four samples). Thus, RCC and normal kidney express a complex set of cadherins.

Introduction

Cadherins constitute a large family of transmembrane glycoproteins. The so-called classical cadherins share a common structure, including a highly conserved cytoplasmic region, a transmembrane region, and an extracellular domain in which homologies define type I and type II cadherins. The most extensively characterized members of this family, E-, P-, and N-cadherins, belong to the type I subgroup (plakoglobin), which in turn interacts with α-catenin that bridges the complex to the actin-based cytoskeleton. The importance of the correct assembly of this complex is exemplified in the fact that cadherins deleted of the 70-carboxyl terminal amino acids are unable to mediate cell adhesion.

In most carcinomas, E-cadherin expression decreases with tumor grade and stage (1). In RCC, however, E-cadherin expression is found infrequently and even low-grade tumors do not express E-cadherin (2). A possible explanation is that the renal proximal tubule epithelium, from which RCCs presumably originate, does not express E-cadherin. Indeed, in normal kidney, E-cadherin is expressed in Bowman’s capsule and in all tubular segments except the proximal convoluted and straight tubules (3). However, using immunohistochemistry we have been able to show α-catenin expression at the intercellular borders in all tubules. Thus, it is likely that functional cadherin-catenin complexes are present in all segments of the nephron. We hypothesized that other classical cadherins form complexes with α-catenin and mediate cell-cell adhesion in RCC. In this article, we used RCC cell lines to characterize RCC for expression of classical cadherins.

Materials and Methods

RCC Cell Lines. RCC cell lines (a kind gift from Dr. L. J. Old, New York, NY) were cultured in RPMI 1640 with 10% FCS and 1% glutamine.

Immunohistochemistry. Cells were grown on glass slides, fixed with 3% paraformaldehyde, and then permeabilized using 0.2% Triton X-100 in PBS. Monoclonal antibody HEC1 (Takara) dilution 1:20 and A-CAM (clone GC4; Sigma) 1:30. Serum of a mouse immunized with a gluthatione S-transferase/α-catenin fusion protein (molecules 454–1981 of human α-catenin), kindly provided by Dr. W. Issacs, Baltimore, MD, was diluted 1:1000. On Western blot, this recognized a single band comigrating with α-catenin.

Immunoprecipitation. Cells were metabolically labeled with [35S]methionine (250 μCi/ml) overnight. Proteins were extracted in 2% Triton X-100, 20 mM Tris (pH 7.4), 150 mM NaCl, 2 mM CaCL, 50 μg/ml leupetin, and 1 mM phenylmethylsulfonyl fluoride. Protein extracts corresponding to 106 cpm were incubated with 1 μl α-catenin mouse antisera. Complexes were collected by protein G-agarose (Oncogene Science) and resolved on a 7% SDS-polyacrylamide gel. Proteins were transferred onto a nitrocellulose membrane. After overnight autoradiography, the membrane was processed for immunoblotting with a pan-cadherin polyclonal antibody diluted 1:1600 (Sigma).

Western Blot. Twenty-five μg of proteins were separated using a 7%; SDS-PAGE and transferred onto nitrocellulose filters. Immunoblots were analyzed using ECL (Amersham) and HEC1 or P-cadherin (Transduction Laboratories), both diluted 1:500.

RT-PCR. Total RNA was extracted from the cell lines with Trizol (Life Technologies, Inc.) and treated with DNase, and the cDNA was synthesized using superscript reverse transcriptase (Life Technologies, Inc.). PCR was carried out using primers described previously (4). PCR products were subsequently run on agarose, and transferred to Biobond N* membranes. Probes corresponded to nucleotides 579–1643 for N-cadherin, 522–2186 for cadherin 4, 25–465 for cadherin 6, and 2759–3256 for cadherin 11 were generated by RT-PCR and labeled with [32P]dATP by random priming. Blots were hybridized overnight in 250 mM NaH2PO4 (pH 7.2), 1 mM EDTA, 1% BSA, and 7% SDS at 65°C and then washed at 65°C successively in 250 mM NaH2PO4 (pH 7.2), 1 mM EDTA, and 1% SDS; in 125 mM NaH2PO4 (pH 7.2), 1 mM EDTA, and 1% SDS; and in 10 mM NaH2PO4 (pH 7.2), 1 mM EDTA, and 1% SDS. To ensure that the expressions we evaluated were not in vitro artifacts, we also included two normal kidneys and four RCC samples.

Results and Discussion

Using immunohistochemistry, we found that all of the cell lines expressed α-catenin at the cell-cell borders. In contrast, E-cadherin was expressed in only six cell lines (Table 1 and Fig. 1). Since in L cells, which do not express any cadherin, α-catenin is unstable, the staining pattern observed here probably reflects the involvement of α-catenin in a protein complex, possibly with a cadherin (5).

To investigate which protein stabilized α-catenin, we immunoprecipitated α-catenin from seven cell lines; one of them (SKRC 59)
The caldesmon-related bands were not noticed possibly reflecting the presence of the various mobilities, but not expressing E-cadherin, representative of the protein products revealed that several caldesmon are present in the four cell lines (five of five clones in SKRC-7, six of eight clones in SKRC-10, four of eight clones in SKRC-8, and five of eight clones in SKRC-24). One of eight clones in SKRC-7 and only one of eight clones in SKRC-10, caldesmon 1 of eight clones in SKRC-7, N-cadherin 1 of eight clones in SKRC-7, N-cadherin 1 of eight clones in SKRC-7, P-cadherin (one of eight clones in SKRC-7), P-cadherin (one of eight clones in SKRC-7), and P-cadherin 1 of eight clones in SKRC-7 were found in the lowest expression level of E- and N-cadherins. We then evaluated the expression of these caldesmons (in four cell lines) at the mRNA level (FV.6 and N-cadherins). A 3.1 kb N-cadherin transcript was present in all of the cell lines. Besides the major transcript, two minor transcripts (3.9 and 2.7 kb) were also present in the two normal kidney and three of four RCCs showing that N-cadherin expression in cell lines is not as uniform as expected (i.e., expression of the different caldesmons is not as well defined). Both mouse and human N-cadherin transcripts are present in the RCC cells. Interestingly, in our study, we found that the major, minor, and four RCC-expressing species are equivalent to the 3.5 kb transcript, whereas the two normal kidney express both 3.5 and 3.5A transcripts. This suggests that there are differential isotypes in the cytoplasm as determined by immunochemistry. The widespread expression of N-cadherin in RCC cell lines was confirmed using immunohistochemistry (Table 1). The fact that two lines (SKR18 and 2B) with clear mRNA expression are negative as determined by RNA. Therefore, we performed immunohistochemistry.
transcriptional regulations. Posttranscriptional down-regulation of N-cadherin has indeed been described during retinal development (9).

Sixteen of the 20 cell lines as well as the 2 normal kidneys and 3 of 4 RCC samples expressed cadherin 6 (Fig. 3). Four major transcripts (9, 4, 3.4, and 2.7 kb) were observed in concordance with earlier studies (10). The 2.7- and 9-kb forms were not found in all cell lines. Moreover, we could clearly see in some cell lines a 4.3-kb transcript which was not described previously by Shimoyama et al. (10). Indeed, this transcript is not visible in the non-retinal cell lines or in the normal organs they have tested, but it can be clearly seen in the KT12 RCC carcinoma they analyzed. Thus, this transcript may be kidney specific.

Using Northern blot, the previously described 3.5-kb cadherin 11 transcript (4) was found in five cell lines (Fig. 3 and Table 1). Both normal kidneys and one of the RCC samples also expressed cadherin 11.

As previously stated, E-cadherin was expressed at the protein level in six cell lines (Table 1). This frequency is in agreement with other studies on RCC samples (19 and 33%). The frequency of negative cell lines (70%) is very high when compared to other cell lines of epithelial origin [e.g., 30% in colon (11), 33% in bladder (12), and 55% in breast (13) cancer cell lines], and is probably related to the fact that proximal epithelial cells, from which RCC originates, do not express E-cadherin.

A weak band, corresponding to a 6-kb cadherin 4 transcript, was seen in only one cell line (Table 1) and one of the RCC; using RT-PCR with specific primers, we could find low levels of cadherin 4 transcript in five more cell lines.

![Figure 3](image-url)  
**Fig. 3.** Expression of cadherins in RCC cell lines examined using Northern blot (N, N-cadherin; 6, cadherin 6; 11, cadherin 11; βA, β-actin) and immunoblot analysis (E, E-cadherin). A 3.4-kb N-cadherin transcript is seen in all of the cell lines including only three lines with a weak signal (SKRC-6, -10, and -33). Two minor transcripts (3.9 and 2.7 kb) can be seen in some cell lines. Cadherin 6 transcripts are detected in 16 of the 20 cell lines. SKRC-10 and -30 have a low mRNA expression level. Four major transcripts (9, 4, 3.4, and 2.7 kb) are observed. Beside these bands, clear 4.3-kb mRNA can be seen in some cell lines. A cadherin 11 transcript is seen corresponding to the size of 3.5 kb in five cell lines (SKRC-10, -28, -12, -35, and -52). The expression is weak in one line (SKRC-12). Note that faint bands related to previous cadherin 6 hybridization can be seen in two cell lines (SKRC-18 and -33). E-cadherin is expressed in six lines as determined using immunoblot.
P-cadherin was not detectable using Western blot in any of the cell lines. Altogether, the RCC cell lines investigated as well as normal kidney displayed a complex pattern of cadherin expression. It is already known that cadherin expression in kidney is highly structured, with differential expression in the various segments of the nephron. For instance, the proximal convoluted tubule and the proximal straight tubule express N-cadherin, the thick ascending limb, the distal convoluted tubule, and the collecting duct express E-cadherin, whereas the thin limb of the Henle loop coexpresses both molecules (3). Based on in situ hybridization studies of the developing kidney, it is likely that cadherins 6 and 11 also have a specific expression pattern in kidney. For cadherin 6, the signal obtained with the Northern blot of normal kidneys was weak when compared to those of the cell lines. This might indicate that in human adult kidney, cadherin 6 expression is also restricted to one particular compartment of the nephron. Indeed, in the rat developing kidney, cadherin 6 expression is restricted to the S-shaped body (the progenitor of, among others, the proximal tubule); no expression was found in the ureter or collecting duct (14).

In the mouse developing kidney, a marked cadherin 11 expression was found in the metanephric mesenchyme surrounding the developing nephron but not in the epithelial nephron (15, 16). Based on the widespread expression of cadherin 11 in mesenchyme (15–17), one can expect that the signal obtained with normal kidney resulted from the expression by the stromal compartment.

It is tempting to relate the cadherin phenotype of RCC to the origin of these tumors along the nephron. The widespread expression of N cadherin (also found by others in RCC samples) supports the generation of mesenchyme by the stromal compartment. For cadherin 6, the signal obtained with the Northern blot of normal kidneys was weak when compared to those of the cell lines.

References